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Another new *Cophixalus* species (Amphibia: Anura: Microhylidae) from western New Guinea

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Abstract. Based on external morphological, anatomical, bioacoustic, and molecular traits, a new species in the microhylid genus *Cophixalus* is described. The new species was discovered in the Fakfak Mountains, northwestern corner of the Bomberai Peninsula, Papua Province, Indonesia. The new taxon is most closely related to the sympatric *Cophixalus tetzlaffi*. It differs from that species in several morphological traits, but primarily by its advertisement call: the new species utters a single peeping note with a mean duration of less than 200 milliseconds, whereas the advertisement call of *C. tetzlaffi* consists of three to four notes, with a mean note duration of more than 400 milliseconds. Molecular data (mitochondrial 16S rRNA) support the determination of the specific distinctness of the new species.

Key words. Anura, Asterophryinae, *Cophixalus*, new species, New Guinea.

INTRODUCTION

Fifty-one species in the microhylid frog genus *Cophixalus* are known at present (Frost 2010). Of these, 14 occur in north-eastern Australia, 30 in Papua New Guinea, three are known only from the Papua Province of Indonesia, three are recorded from both Papua New Guinea and the Papua Province of Indonesia, and one species seems to be endemic to the Island of Halmahera, located about 300 km west of the western tip of New Guinea. Although many new *Cophixalus* species are expected to be described also from western New Guinea, the distribution centre of the genus seems to be clearly in eastern New Guinea and north-eastern Australia. *Cophixalus montanus* has been known since 1895 from Halmahera, and the detection of three new species in the western part of New Guinea (on Yapen Island, on the Wandammen Peninsula, and on the Bomberai Peninsula) came as a surprise (Günther 2003, 2006).

Here I describe another new species from the Fakfak Mountains on the Bomberai Peninsula (located on the "throat" of the Vogelkop) found during an expedition in September 2008. Moreover, a population of *Cophixalus tridactylus* and one specimen of a second undescribed species was observed there. Consequently, at least four *Cophixalus* species occur syntopically in the middle and higher elevations (400–1000 m above sea level, a.s.l.) of the Fakfak Mountains.

MATERIAL AND METHODS

Most frogs were collected at night after locating them by their advertisement calls. Some specimens were photographed in life the next day and all specimens were anaesthetized with chlorobutanol and subsequently fixed in 2 % formalin. Tissue probes from thigh muscle were taken from some frogs and stored in about 96 % ethanol to enable DNA sequencing, before fixing the animals in formalin. All specimens were transferred to 75 % ethanol later in the Berlin museum. One specimen was cleared and stained as an osteological preparation according to a method modified from Dingerkus & Uhler (1977).

The following measurements were taken with a digital calliper (> 10 mm) or with a binocular dissecting microscope fitted with an ocular micrometer (< 10 mm) to the nearest 0.1 mm:

SUL – snout-urostyle length: from tip of snout to distal tip of urostyle-bone; SUL is about one to two mm shorter than the snout-vent length (SVL). As the measurement error is higher in the latter, I prefer to use the former. In general, both measurements are more or less identical and are used interchangeably in this paper.

TL – tibia length: external distance between knee and ankle;

TaL – length of tarsus: external distance, tarsal and ankle joints held at a right angle;

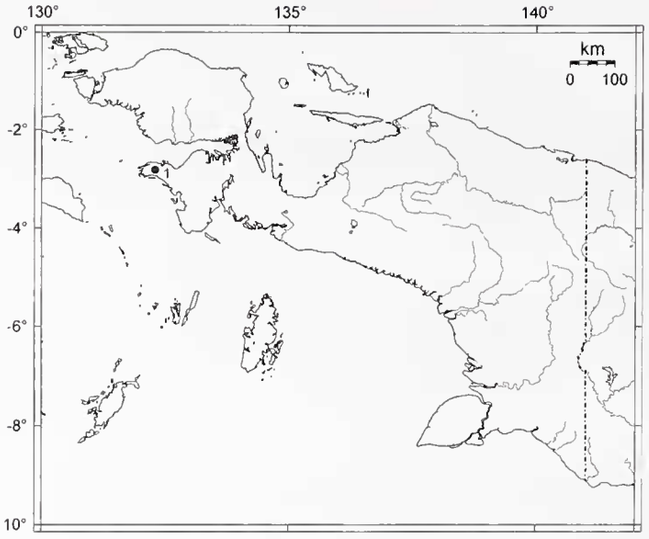

Fig. 1. Map of the western part of New Guinea with type locality (1).

Fig. 2. Holotype of *Cophixalus monosyllabus* sp. n. head in lateral view (above); head in dorsal view (below).

T4L – length of fourth toe: from tip of toe to proximal end of inner metatarsal tubercle;
T4D – transverse diameter of disc of fourth toe;
F3L – length of third toe;
F3D – transverse diameter of disc of third finger;
F1D – transverse diameter of disc of first finger;
T1D – transverse diameter of disc of first toe;
HL – head length: from tip of snout to posterior margin of tympanum;
HW – head width, taken in the region of the tympana;
SL – snout length: from an imaginary line that connects the centres of eyes to tip of snout;
END – distance from anterior corner of orbital opening to centre of naris;
IND – internarial distance between centres of nares;
ED – eye diameter: from anterior to posterior corner of orbital opening;
TyD – horizontal diameter of tympanum.

Advertisement calls were recorded under natural conditions with a Sony Digital Audio Tape (DAT) Walkman TCD-D 100 and a Sennheiser microphone MKE 300 and analysed with Avisoft-SAS Lab Pro software. All specimens are currently stored in the Museum für Naturkunde Berlin (ZMB) and bear registration numbers of that institution. Part of the type series will later be transferred to the Museum Zoologicum Bogoriense (MZB).

RESULTS AND DISCUSSION

Cophixalus monosyllabus sp. n.

Holotype. ZMB 74993 (field number, FN: RG 7888) ; adult male collected by R. Günther and A. Piahar 6 km direct line NNE of Fakfak town, near the Fakfak-Kokas road, Bomberai Peninsula (neck of Vogelkop), Papua Province, Indonesia, 2°53'S and 132°18'E, elevation 500 m a.s.l., 9 September 2008 (Fig. 1).

Paratypes. ZMB 74994 (FN: RG 7889), ZMB 74995 (FN: RG 7890), ZMB 74996 (FN: RG 7912), ZMB 74997 (FN: RG 7915), ZMB 74998 (FN: RG 7916), ZMB 74999 (FN: RG 7926), ZMB 75000 (FN: RG 7927), ZMB 75001 (FN: RG 7951), ZMB 75002 (FN: RG 7952). ZMB 74997 is now an osteological preparation. All nine paratypes are males. They were collected from 9 to 14 September 2008 along the Fakfak-Kokas road in the southern part of the Fakfak Mountains, at elevations of from 400 to 700 m a.s.l. Collectors were R. Günther, M. Kapisa, and A. and F. Piahar.

Table 1. Body measurements and body ratios of the type series of *Cophixalus monosyllabus* sp. n. ZMB-No are the inventory numbers of the Museum für Naturkunde Berlin, FN are the field numbers of the author, SD indicates the standard deviation. ZMB 74993 is the holotype; ZMB 74997 is now an osteological preparation. All specimens are adult males. All measurements are in mm; abbreviations are explained in "Material and methods".

ZMB-No	74993	74994	74995	74996	74997	74998	74999	75000	75001	75002	mean	SD
FN	7888	7889	7890	7912	7915	7916	7926	7927	7951	7952	22.9	1.04
SUL	22.8	23.1	24.1	22.5	22.4	23.6	20.6	22.8	24.3	23.0		
TL	10.9	11.7	11.4	11.5	11.3	11.5	10.1	11.7	12.3	11.7		
TaL	7.4	7.2	7.1	6.8	6.9	7.2	7.2	7.6	7.3	7.3		
T4L	11.5	11.6	11.7	11.6	11.2	11.1	10.5	11.1	12.5	10.9		
T4D	1.3	1.25	1.4	1.0	1.3	1.3	1.0	1.2	1.35	1.25		
T1D	0.7	0.65	0.75	0.5	0.7	0.6	0.45	0.7	0.6	0.6		
F3L	6.0	6.4	6.8	6.4	6.1	6.5	5.5	5.9	6.8	6.0		
F3D	1.4	1.4	1.7	1.45	1.5	1.3	1.25	1.5	1.5	1.4		
F1D	0.6	0.45	0.50	0.50	0.5	0.4	0.45	0.5	0.5	0.5		
HL	7.5	7.2	8.2	7.1	7.3	8.0	6.8	7.3	7.5	7.5		
HW	8.5	9.0	9.1	9.0	9.5	9.8	8.5	9.0	9.6	9.6		
SL	3.2	3.3	3.6	3.3	3.5	3.4	3.2	3.3	3.5	3.6		
END	2.2	2.1	2.5	2.0	2.1	2.2	2.0	2.1	2.2	2.0		
IND	2.3	2.5	2.5	2.3	2.2	2.4	2.2	2.5	2.5	2.25		
ED	2.8	2.9	3.1	2.5	2.9	2.8	2.7	2.8	3.0	3.0		
TyD	1.2	1.0	1.2	1.0	1.0	1.1	1.0	0.9	1.0	1.0		
TL/SUL	0.48	0.51	0.47	0.51	0.50	0.49	0.49	0.51	0.51	0.51	0.50	0.051
TaL/SUL	0.32	0.31	0.29	0.30	0.31	0.31	0.35	0.33	0.30	0.32	0.31	0.017
T4L/SUL	0.50	0.50	0.49	0.52	0.50	0.47	0.51	0.49	0.51	0.47	0.50	0.016
F3L/SUL	0.26	0.28	0.28	0.28	0.27	0.28	0.27	0.26	0.28	0.26	0.27	0.009
F3D/SUL	0.061	0.060	0.071	0.064	0.067	0.055	0.061	0.066	0.062	0.061	0.063	0.004
F1D/SUL	0.026	0.019	0.021	0.022	0.031	0.017	0.022	0.022	0.021	0.022	0.021	0.002
T4D/SUL	0.057	0.054	0.058	0.044	0.058	0.055	0.049	0.053	0.056	0.054	0.054	0.004
T1D/SUL	0.031	0.028	0.031	0.022	0.031	0.025	0.022	0.031	0.025	0.026	0.027	0.004
HL/SUL	0.33	0.31	0.34	0.32	0.33	0.34	0.33	0.32	0.31	0.33	0.33	0.011
HW/SUL	0.37	0.39	0.38	0.40	0.42	0.42	0.41	0.39	0.40	0.42	0.40	0.018
HL/HW	0.88	0.80	0.90	0.79	0.77	0.82	0.80	0.81	0.78	0.78	0.81	0.043
END/IND	0.96	0.84	0.84	0.87	0.95	0.92	0.91	0.84	0.88	0.89	0.89	0.044
ED/SUL	0.123	0.125	0.129	0.111	0.129	0.119	0.131	0.123	0.120	0.130	0.124	0.006
TyD/ED	0.43	0.34	0.39	0.40	0.34	0.39	0.37	0.32	0.33	0.33	0.36	0.037
SL/SUL	0.140	0.143	0.149	0.147	0.156	0.144	0.155	0.145	0.141	0.156	0.148	0.006

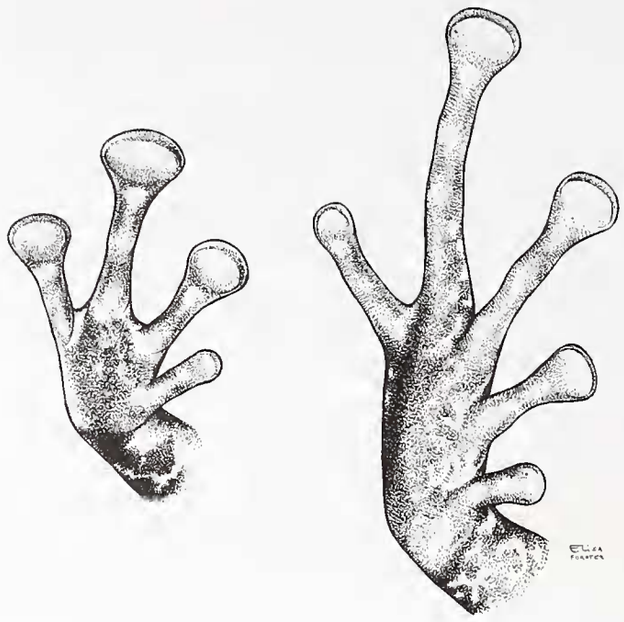

Fig. 3. Holotype of *Cophixalus monosyllabus* sp. n. ventral view of right hand (left); ventral view of right foot (right).

Diagnosis. With a snout-urostyle length of from 20.6 to 24.3 mm in ten adult males, the new species belongs to the middle-sized species of the genus. It is obviously a sister species of the sympatric *Cophixalus tetzlaffi* and differs from all other species in the same characters as the latter. The new species differs from *C. tetzlaffi,* among others, by its larger body size, its wider finger and toe discs, and its advertisement call which consists of only one peeping syllable (note) that lasts, on average, 196 milliseconds (ms). In contrast, the advertisement call of *C. tetzlaffi* consists of three to four peeping notes, with a mean note length of more than 400 ms.

Description of the holotype. For measurements see Table 1. Head broader than long (HL/HW ratio 0.88), canthus rostralis roundish; loreal region straight; snout slightly protruding in profile (Fig. 2, above) and rounded in dorsal view (Fig. 2, below); horizontal eye diameter greater than eye-naris distance; borders of tympanum scarcely visible, its size less than half of the eye diameter (TyD/ED 0.43), no supratympanic fold; internarial distance slightly greater than distance between eye and naris (END/IND 0.96); tongue large, posteriorly broadened and without posterior notch, its posterior and lateral margins free; a strongly serrated fold present in front of the pharynx; long

Fig. 4. Dorsolateral view of a more brownish coloured paratype of *Cophixalus monosyllabus* sp. n. (ZMB 74995).

Fig. 5. Dorsolateral view of a more greyish coloured paratype of *Cophixalus monosyllabus* sp. n. (ZMB 74999).

Fig. 6. Ventral view of a paratype of *Cophixalus monosyllabus* sp. n. (ZMB 74995).

slits on both sides of the tongue are entrances to a subgular vocal sac. Legs moderately long, no webs between fingers or toes (Fig. 3); tips of fingers wider than tips of toes, first finger much smaller than other fingers, its tip only scarcely wider than the penultimate phalanx; relative length of fingers 3>2=4>1; third toe clearly longer than the fifth, tip of first toe slightly smaller than tip of the fifth toe, tips of remaining toes clearly wider than that of first and fifth toe; all finger and toe tips with terminal grooves; relative length of toes 4>3>5>2>1, all subarticular tubercles as well as metatarsal and metacarpal tubercles not or only scarcely developed. With exception of some tubercles on flanks, all dorsal, lateral, and ventral surfaces smooth.

Dorsum light brown and clearly demarcated against dark brown upper flanks, dorsal surfaces of legs non-uniform brown, chevron-shaped mark in scapular region, dorsal surface of snout lighter than remaining body; dorsal surfaces of fingers and toes with yellowish, light brown, and dark brown pattern; lateral and dorsolateral flanks with longitudinal rows of blackish spots, a conspicuous blackish spot present also above insertion of forelcg and bchind eye; loreal region, tip of snout, and region below and behind eye and underneath tympanum blackish (black face mask); ventral surface of forelegs yellowish with irregu-

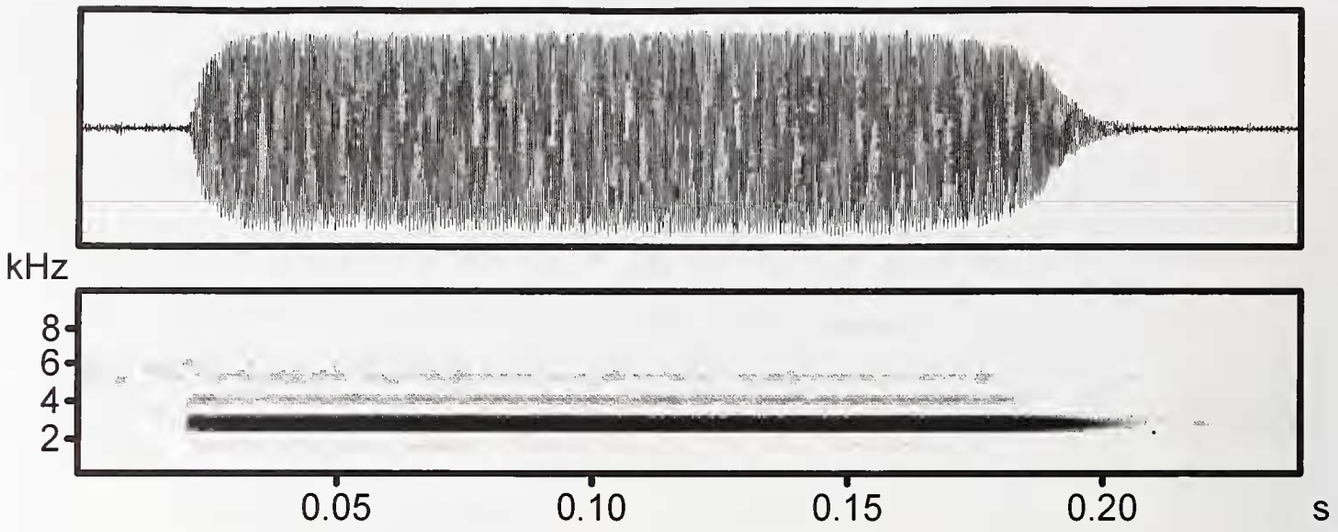

Fig. 7. Wave form (above) and spectrogram (below) of an advertisement call of *Cophixalus monosyllabus* sp. n..

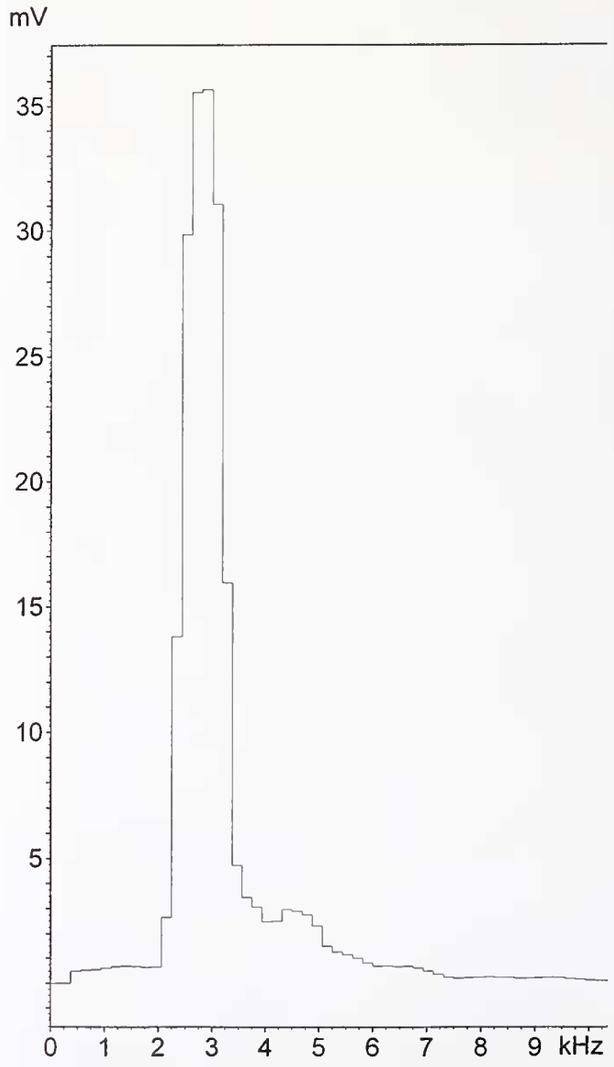

Fig. 8. Power spectrum of an advertisement call of *Cophixalus monosyllabus* sp. n.

lar dark brown flecks, ventral surface of hind legs also yellowish with brown flecks but the latter less intense than on forelegs (ventral skin and muscle tissue of the right thigh was removed for biochemical studies); belly, chest and throat yellowish with brown pigmentation, pigmentation most intense on throat and chest and least intense on abdomen; region around anal opening blackish and region from behind eye, through tympanum, and up to upper arm whitish.

Variation in the type series: Mensural variation for the type series is shown in Table 1. The basic colour and colour pattern elements of all preserved types are fairly uniform and very much resemble those of the holotype. Characteristic elements are a light brown dorsum, which is differentiated from dark brown upper flanks, a dark brown and irregularly pronounced interocular band, a dark chevron or W-shaped mark in the scapular region, a dark face mask, a blackish spot behind eye and above insertion of fore leg, a blackish throat which fades posteriorly into a diffuse dark brown reticulum, and a pale dorsal surface of the snout which is the palest part of all the dorsal surfaces. Only one specimen (ZMB 74996) has a whitish middorsal line from snout tip to anal opening and which continues on to the posterior thighs.

The basic colour in life varies from crème or grey to light brown. Dorsum rather uniform brownish (Fig. 4) or grey (Fig. 5); conspicuous is a blackish or dark brown chevron or W-shaped mark in the scapular region, an irregular dark brown interocular band between posterior parts of eyes, and a crème or light brown coloured dorsal part of snout. Lower flanks are mostly lighter than the remaining lateral areas (Fig. 4). Upper flanks may be of nearly the same

Fig. 9. Habitat of *Cophixalus monosyllabus* sp. n. in the Fakfak Mountains on the Bomberai Peninsula, 700 m a.s.l.

brown or grey colour as on the dorsum. Conspicuous are a blackish spot behind the eye, another blackish spot above the foreleg insertion, and some blackish spots at the border between the dorsum and flanks. It is notable that the dorsum in all preserved specimens is clearly lighter than the upper flanks, whereas the dorsum and upper flanks in most living specimens differed in colour only slightly. Loreal region in all specimens entirely or predominantly black. In some specimens this black area continues to below the eye and extends up to the upper arm, in others this black area ends below the eye. The inner margin of the "upper eyelid" is whitish in most specimens, this colour merging in a broad and light postocular band.

While there are no or only a few tubercles in the preserved specimens, most living specimens exhibited tubercles on the flanks and extremities (rarely on the dorsum). Many of these tubercles have a blackish base and a orange-red cap. Most, and the largest, tubercles are arranged in dorsolateral rows. The yellowish spot posterior of the chevron sign in ZMB 74999 (Fig. 5), which faded to a white spot in preservative, is obviously an exception. Orange-red areas were also found on the forelegs of some specimens. The fine whitish middorsal line in the living specimen in Fig. 5 disappeared completely in fixative. Dorsal sides of legs similarly coloured as other dorsal and dorsolateral body parts. Ventral sides of forearms crème-coloured, its anterior and posterior part covered with irregular dark spots. Throat and chest in all specimens darker than on the remaining ventral surfaces. These dark ventral areas are solidly or discontinuously black or dark brown. Abdomen and ventral sides of hind legs show greater light areas covered by a more or less dense reticulum of grey-brown. Weakest pigmentation was commonly on the posterior abdomen (Fig. 6). Iris yellow-red and nerved by a dense net of blackish lines.

Osteology. One cartilage-bone preparation (ZMB 74997) did not show remarkable differences from that of *Cophixalus tetzlaffi* (see Günther 2003).

Vocalisation. Most calling activities were recorded during rain and damp weather from dusk to 9 p.m. All calls were recorded at temperatures of approximately 21°C. Calls are uttered in series lasting several minutes. The shortest time between two successive calls was about 3 s. Each call consists of a single unpulsed and finely tuned note (Fig. 7). Fifty-six calls (notes) from two males had

Fig. 10. An undescribed *Cophixalus* species from the Fakfak Mountains, with a 16.9 mm snout-urostyle-length, which at first glance resembles *Cophixalus misimae* recently described by Richards & Oliver (2007) from Misima Island, Louisiade Archipelago, Papua New Guinea.

a mean length of 196 ms, with a minimum of 173 ms and a maximum of 224 ms. Most notes start with a sharp increase in amplitude, and the sound volume may remain constant during the entire note but may also change, with the greatest sound volume mostly near the end. The end of the note occurs more gradually and its exact cessation is fairly difficult to identify (Fig. 7). The dominant frequency is approximately 2.8 kHz (Fig. 8), the fundamental frequency is approximately 1.4 kHz, and the first (and most pronounced) upper harmonic band is at about 4.2 kHz.

Distribution. The new species lives on slopes and in valleys of the southern part of the Fakfak Mountains. We found it along the Fakfak town-Kokas road at elevations of from 250 to 700 m a.s.l. Whether it also occurs in the northern part of the Fakfak Mountains remains to be determined.

Habitat and habits. *Cophixalus monosyllabus* sp. n. lives mostly in the understory (bushes, young trees, and herbs) of taller trees but also in shrubbery without a canopy cover (Fig. 9). The frogs perched mainly on or between living or dead leaves at heights of from one to three meters above the ground. The species is common: we heard several hundred males during a walk of three kilometres along the Fakfak-Kokas road. Some males called at distances of only two m from one another. At favoured places about ten males could be heard calling from one point. For biogeographical reasons it seems worthwhile to mention that at elevations of between 500 and 700 m a.s.l., *C. monosyllabus* sp. n. occurs syntopically with *C. tetzlaffi*, *C. tridactylus,* and another obviously new *Cophixalus* species

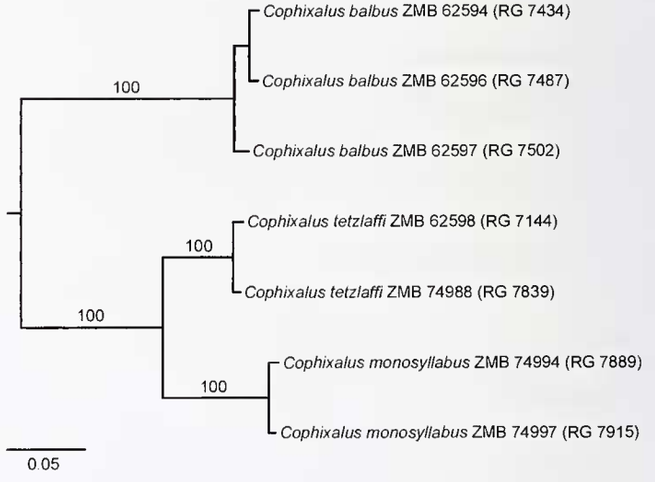

Fig. 11. Bayesian inference phylogram of 16S rRNA. Numbers on branches denote posterior probabilities.

(Fig. 10). Ecological differences between these four species are the following: *C. monosyllabus* sp. n. occurs at from 250 to 700 m a.s.l. and its calling sites are at between one and three metres above ground; *C. tetzlaffi* occurs at from 400 to 900 m a.s.l. (top of the mountains) and its calling sites are on structures up to one m above the ground; *C. tridactylus* was found at from 500 to 900 m a.s.l. and its calling sites are on the ground; and the obviously new species was found at 860 m a.s.l. in humus soil below the ground surface.

Etymology. The Latin word "monosyllabus" is derived from the Greek composite adjective "monosyllabos" meaning one syllable or monosyllabic, and refers to the advertisement call of the new species which consists of only one uniform note. I dedicate this new species to my dear colleague of many years, Prof. Dr. Wolfgang Böhme, to acknowledge his extraordinary contributions to herpetological science and on the occasion of his retirement from official service, although it is well known that Wolfgang is by no means monosyllabic but rather is very eloquent.

Molecular evidence. According to B. Stelbrink and T. von Rintelen (pers. comm., July 2010) DNA isolation and PCR were done using the protocol of Köhler & Günther (2008). Forward and reverse strands were aligned using CodonCode Aligner v. 3.0.3 (CodonCode Corporation, Dedham, MA, USA) and corrected by eye. Sequences were aligned using MAFFT (Katoh & Toh 2008) and optimized using ALISCORE (Misof & Misof 2009). Phylogenetic analysis (Bayesian inference) was accomplished as conducted by Günther et al (2010).

The analysis of 480 base pairs of the 16S rRNA gene revealed that *Cophixalus monosyllabus* sp. n. is clearly a sister species of *C. tetzlaffi* and both are a sister clade of *C. balbus* (Fig. 11). *C. tridactylus* and *C. humicola* appear more distant in the molecular tree (see also Köhler & Günther 2008), and indicate that the present genus *Cophixalus* most probably is polyphyletic. The genetic distance (uncorrected p-distance) between *C. monosyllabus* sp. n. and *C. tetzlaffi* is 4.3 % for the 16S rRNA gene.

Comparison with other species. *Cophixalus monosyllabus* sp. n. is distinct from other *Cophixalus* species, described up to the year 2003, in the same characters as is *C. tetzlaffi* (Günther 2003). All 16 *Cophixalus* species described after 2003 (Hoskin 2004; Kraus & Allison 2006, 2009; Günther 2006; Richards & Oliver 2007) differ clearly from *C. monosyllabus* sp. n. in body size and also in their advertisement calls. The only species with which *C. monosyllabus* sp. n. can be confused morphologically is *C. tetzlaffi*, especially as both species occur syntopically.

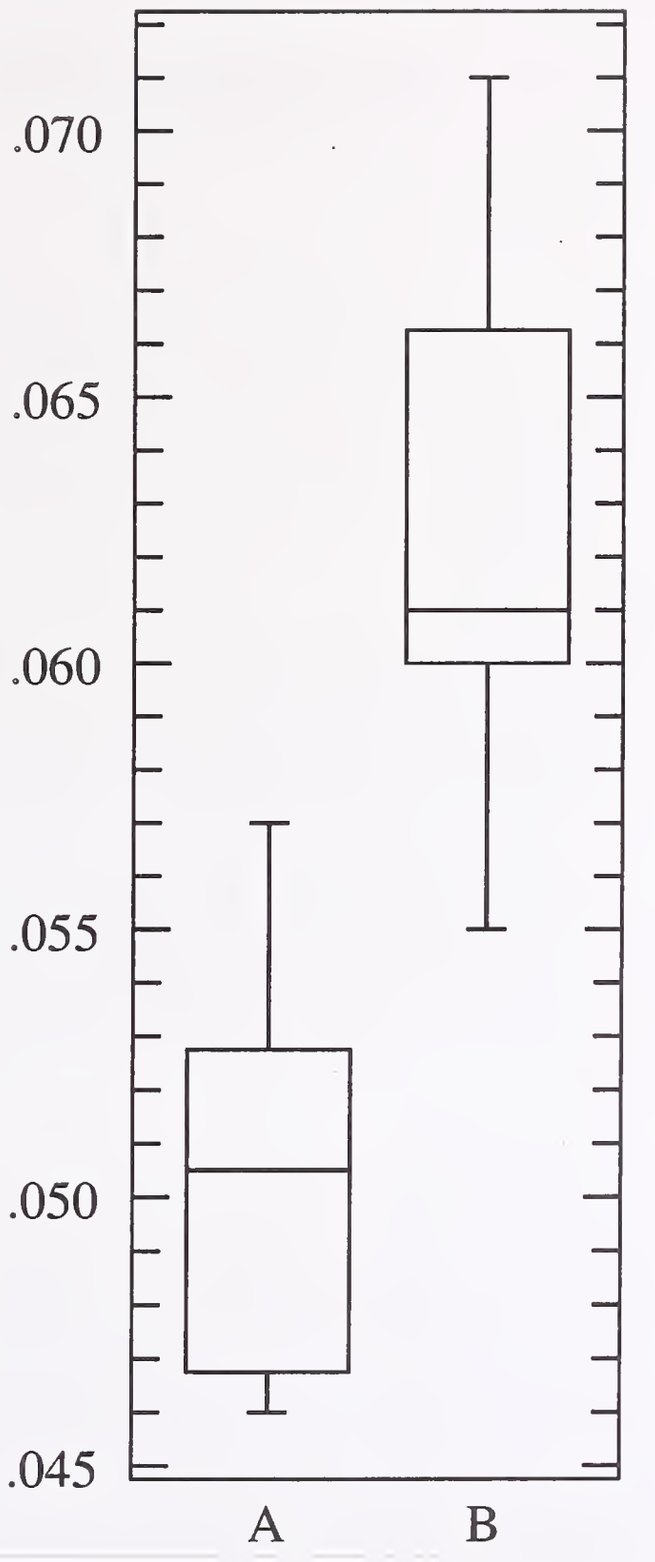

Fig. 12. Box-Whisker-Plot of the ratio "diameter of disc of fourth toe/snout-urostyle-length" (F3D/SUL) in *Cophixalus tetzlaffi* (A) compared to that of *Cophixalus monosyllabus* sp. n. (B).

I compared the measurements of ten male *C. monosyllabus* sp. n. with that of eight male *C. tetzlaffi* and found the following differences: with a mean body size (SUL) of 23.0 mm (range 20.6–24.3 mm), *C. monosyllabus* sp. n. is somewhat larger than C. *tetzlaffi* (mean 21.4 mm, range 19.5–22.6 mm), Student´s t-test revealed a significant difference with t=2.98 and P=0.0046 in this character; *C. monosyllabus* sp. n. has significantly shorter tibiae than *C. tetzlaffi* (mean of TL/SUL in *C. monosyllabus* sp. n. 0.50, that in *C. tetzlaffi* 0.52, t= 3.39, P=0.0019); *C. monosyllabus* sp. n. has a longer third finger than *C. tetzlaffi* (mean of F3L/SUL in the former 0.27, in the latter 0.26, t=3.01, P=0.0041; *C. monosyllabus* sp. n. has a wider terminal disc on the fourth toe than C. *tetzlaffi* (mean of T4D/SUL in the former 0.054, in the latter 0.046, t=3.11, P=0.0094); *C. monosyllabus* sp. n. has a wider terminal disc on first toe than *C. tetzlaffi* (mean of T1D/SUL in the former 0.027, in the latter 0.018, t=5.63, P=0.00002); *C. monosyllabus* sp. n. has a wider terminal disc on first finger than *C. tetzlaffi* (mean of F1D/SUL in the former 0.021, in the latter 0.015, t=5.36, P=0.00003) and, most significantly, *C. monosyllabus* sp. n. has a wider terminal disc on the third finger than *C. tetzlaffi* (mean of F3D/SUL in the former 0.063, and in the latter 0.051, t=6.14, P=0.000007) (Fig. 12). There are continuous dorsolateral skin ridges in *C. tetzlaffi*, but discontinuous dorsolateral skin glands in *C. monosyllabus* sp. n.

Apart from these morphological differences, and most important for species differentiation, are the advertisement calls: *C. monosyllabus* sp. n. utters a single peeping note with a mean duration of 196 ms (range 173–224 ms), while the call of *C. tetzlaffi* consists of three to four peeping notes with a mean note duration of 422 ms (range 347–518 ms).

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